This international symposium dealt with ion channels in pain and neuroprotection. It consisted of five sessions, each with four to five presentations, three plenary lectures, and 50 posters. It was sponsored and organized by Roche Bioscience with additional support from Astra, Pfizer, Abbot, Merck, Novartis, Rhone-Poulenc and Wyeth. Approximately 400 academic and industrial scientists attended this symposium, which was held at the Ritz-Carlton Hotel in San Francisco, CA.

ORAL PRESENTATIONS

The session on sodium channels was opened by John C. Hunter (Roche Bioscience, Palo Alto, CA). He emphasized in his introduction that alterations in the expression and distribution of tetrodotoxin-resistant (TTX-RI) sodium channel subtypes (PN3/SNS) may contribute to abnormal processing of nociceptive information, and the inhibitors of these subtypes may be useful in the therapy of chronic inflammation. Jim R. Elliott (Univ. of Dundee, Scotland, U.K.) discussed the properties and function of sodium channels in sensory neurons as well as the therapeutic potential of modulators of these channels. TTX-RI currents are likely to support firing in neurons depolarized by high external potassium in damaged tissues. Alan L. Goldin (Univ. of California at Irvine, CA) discussed the molecular and functional diversity of sodium channels. Three different types of voltage-gated sodium channels were identified. Type 1 channels have eight isoforms (Nav \( 1_{1-8} \)). The following isoforms may have clinical relevance: Nav \( 1_2 \), in epilepsy; Nav \( 1_4 \), in periodic paralysis; Nav \( 1_5 \), in long QT; Nav \( 1_6 \), in motorendplate disease in mice; and Nav \( 1_8 \) (PN3), in neuropathic pain. Three isoforms of the type 1 channel are expressed in the Purkinje cells of the adult central nervous system (1, 2 and Scn 8a). Scn 8a channel is likely to be responsible for the sodium conductance that underlies the repetitive firing of action potentials in Purkinje neurons. Lakshmi Sangameswaran (Univ. of Ar-
izona, Tuscon, AZ) further characterized novel sodium channels in peripheral sensory neurons. The following sodium channels cDNAs have been isolated by homologous cloning procedures: PN1, PN3/SNS, PN4/NaCh6/Scn8, PN5/NaNS/SNS2, rB1, rBIII, glial, and atypical sodium channels. PN1 and PN4 are TTX-sensitive and are expressed in DRG neurons. PN3 is slowly inactivated and is relatively insensitive to TTX (IC$_{50}$ = 100 µM), and it may play a role in the high-frequency burst firing associated with neuropathic pain. In his lecture, John C. Hunter (Roche Bioscience, Palo Alto, CA) discussed the evidence supporting the importance of the PN3/SNS sodium channel in the mediation of chronic pain and inflammation. This channel is preferentially located in the sensory afferent neurons (DRGs). The expression of this channel is increased following complete Freund's adjuvant-induced chronic inflammation. Antisense oligodeoxynucleotides (ODN) to PN3 reverse tactile allodynia and thermal hyperalgesia in chronic inflammation models but not in carrageenan-induced acute inflammation. Drugs targeted at this channel are expected to have a better safety profile than less-specific analgesic agents. Miriam H. Meisler (Univ. of Michigan, Ann Arbor, MI) generated transgenic mice with a mutation in SCN2A (rbII) and a dominant seizure disorder. Meisler is currently screening human patients with inherited ataxia and dystonia for SXN8a mutations.

In the first plenary lecture, William B. Catterall (Univ. of Washington, Seattle, WA) emphasized that the β1 and β2 subunits of voltage-gated sodium channels modulate channel expression and gating. They interact with the α-subunit through their extracellular domain. α-Subunits consist of four homologous domains, each with six transmembrane helices (S$_{1-6}$). Positively charged residues in the S$_{4}$ segment serve as voltage sensors to activate the channels. The inactivation is achieved by closing the inactivation gate in the loop between domains III and IV of the α-subunit. Local anesthetics bind to a receptor site in the S$_{6}$ segment of domain IV. α-Scorpion toxin blocks inactivation, whereas β-scorpion toxin enhances activation. Most peptide toxins are likely to act by a mechanism that involves voltage-sensor trapping.

The session on calcium channels was chaired by Richard W. Tsien (Stanford Univ., Palo Alto, CA). The first speaker, Terry P. Snutch (Univ. of Brit. Columbia, Vancouver, Canada) discussed G-protein and protein kinase C-dependent modulation of the subtypes of neuronal calcium channels implicated in pain modulation. N-type and P/Q-type calcium channels (expressed in Xenopus oocytes or HEK 293 cells) are inhibited by opioids. Their slow activation kinetics and inhibitory effect are G-protein-dependent. G$\gamma$ binds to the domain I–II linker of the α$_{1}$-subunit of calcium channel to inhibit activation of opioid receptors. The residues in the domain I–II linker also inhibit G-protein interaction through phosphorylation by protein kinase C. The transcription of syntaxin-1A is activated by calcium influx via P/Q channels. Richard W. Tsien and his associates studied modulation of N- and P/Q-type calcium channels by syntaxin 1A (S1A). The availability of these channels (expressed in Xenopus oocytes) is decreased by S1A. The slow inactivation of N-type channels is accentuated by S1A, whereas S1A has little or no effect on the activation of the same channels. In the oocyte system botulinum toxin C$_{1}$ (BoTxC$_{1}$) reduces the effects of S1A on calcium channel activity. In synaptosomes late, but not initial, entry of calcium (caused by K$^{+}$-induced depolarization) is augmented by syntaxin cleavage. In L-type channels, calmodulin is a critical sensor for activation and facilitation.
Calmodulin interacts with an isoleucine-glutamine motif in the cytoplasmic C-terminal tail of the \( \alpha_{1C} \) subunit.

**B. M. Olivera** (Univ. of Utah, Salt Lake City, UT) estimated that the venoms of cone snails contain up to 50,000 different small peptides that target ion channels. \( \alpha \)-Conotoxins target voltage-gated calcium channels. \( \delta \)-Conotoxins increase and \( \mu \)-conotoxins decrease conductance in sodium channels. Conantokins block calcium entry through NMDA-receptor subtypes. Conantokins are being currently evaluated by Cognetix for various central nervous system indications. Some conantokins have a higher protective index than MK 801.

The session on proton-sensitive and glutamatergic channels was opened by **Michel Lazdunski** (IPMC-CNRS, Valbonne, France), who discussed acid-activated and mechano-gated \( \text{Na}^+ \) and \( \text{K}^+ \) channels. Amiloride-sensitive epithelium sodium channels were cloned. They contain \( \alpha \), \( \beta \), and \( \gamma \) subunits and two transmembrane domains, are highly regulated, and their mutation can lead to hypertension. More recently discovered \( \text{H}^+ \)-gated sodium channels include ASIC and DRASIC. ASIC is expressed in the brain and dorsal root ganglion cells (DRGs). DRASIC is present primarily in DRGs. ASIC rapidly activates and inactivates, while DRASIC has both, rapidly and slowly activating components. There are at least four different ASICs. ASIC I is expressed throughout the brain, is possibly involved in the modulation of pain, and is sensitive to amiloride. Its pH dependency is controlled by a glycine residue located just before the second domain. Two new mechano-sensitive \( \text{K}^+ \) channels have been recently cloned: TREK and TRAAK. They are involved in the conversion of mechanical activity into an electric signal. TREK is blocked by acidic pH. TRAAK is an acid-activated channel. Both channels are highly expressed in the central nervous system. They are activated also by polyunsaturated fatty acids. TREK-1 subtype is the target of volatile anesthetics (e.g., chloroform, isoflurane, halothane). The C-terminal region of TREK-1 is critical to activate the channel by anesthetics. The title of **Phillipe Séguéla’s** (McGill Univ., Montreal, Quebec, Canada) presentation was “Two-Transmembrane-Domain Ionotropic Receptors in Sensory Pathways.” He compared the properties of ASICs with those of ATP-gated channels (P2X receptors). They had no significant homology, but they had multiple common features. Both channels are abundantly expressed in primary sensory neurons. Their amino- and carboxy-terminal domains are located intracellularly, and their two transmembrane domains are separated by a cysteine-rich extracellular loop. ASICs, as well as P2X receptors, can serve as detectors of substances elevated in inflammation, hypoxia, and trauma. **Stuart Bevan** (Novartis Institute for Medical Sciences, London, U.K.) discussed proton-activated ion channels in sensory neurons. According to Bevan acidic solutions activate two classes of currents in rat sensory neurons. One of the currents rapidly activates and deactivates and is found in the majority of DRG neurons, while the second, more persistent current is found only in a small number of the DRG neurons. The second current is similar to the current activated by capsaicin. Capsaicin receptors and currents, but not acid-activated currents, are blocked by capsazepine. **M. Caterina et al.** (Univ. of California at San Francisco, CA) further analyzed ion channels in sensory neurons. They identified cDNA that encodes for the capsaicin (or vanilloid) receptor (VR1). This receptor is expressed in small- to medium-size neurons in the dorsal route, trigeminal, and nodose ganglia, and it transmits chemical and thermal stimuli. It can be activated by thermal or acidic stimuli. The VR1 receptor is likely to be involved in neurogenic inflammation. **R. A. North** (Univ. of Shef-
field, U.K.) discussed P2-purinergic receptors. ATP and related nucleotides act at P2X-(ionotropic) and P2Y- (metabotropic) receptors. P2X receptors have seven subtypes (P2X1–7). P2X1 subtypes are expressed in vascular smooth muscle. All subtypes are expressed in the nervous system. The permeation properties of P2X receptors can be changed with the sustained application of an agonist.

Another session on glutamatergic channels was chaired by John A. Kemp (Hoffmann-La Roche, Basel, Switzerland). The first speaker was Stephen F. Heinemann (Salk Institute, San Diego, CA). He and his colleagues successfully developed kainate receptor-deficient mice. Point mutations were produced in each of the known kainate receptor genes: GluR 5, Glur 6, GluR 7, KA-1, and KA-2. The mutant mice were viable. GluR 6-deficient mice have reduced locomotor activity and are resistant to kainate-induced seizures. John A. Kemp spoke about NMDA-receptor subtypes, their subunit composition, and their molecular pharmacology. They contain combinations of NR1 and NR2 subunits that are usually coexpressed. It appears that some native receptors may contain more than one type of NR2 subunit. The subtypes of NMDA receptors are attractive targets for therapeutic intervention, and Hoffmann-La Roche developed selective NR2 subtype-selective antagonists. A. H. Dickenson (University College, London, U.K.) discussed the role of glutamatergic channels in central sensitization and prolonged nociception. Excitatory aminoacid receptors play different roles in nociception. AMPA receptors appear to be involved in the transmission of sensory information, while kainate receptors are more important in noxious transmission after inflammation. The NMDA receptor is important in the generation of central sensitization. The repeated stimulation of C fibers enhances NMDA-dependent nociceptive transmission through the spinal cord. NMDA receptors induce and maintain noxious transmission after inflammation, ischemia, and nerve injury. N-type calcium channel antagonists reduce noxious activity in normal animals and are particularly effective in models of inflammation and neuropathy. An increased entry of calcium into neurons after NMDA-receptor activation can activate nitric acid synthase and generate NO, which contributes to the maintenance of central sensitization. Glycine site antagonists are highly effective in protecting the spinal cord. Memantine is a more effective blocker in ischemic than normal tissue. Different calcium channels are involved in different phases of inflammation.

The second plenary lecture entitled “The GABA-A Receptor: from Gene to Function” was delivered by Paul J. Whiting (Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex, U.K.). Many GABA_A receptor genes (α1–α6; β1–β3; γ1–γ3; δ; ε; θ) have been identified. Their polypeptides co-assemble to form a number of receptor subunits (e.g., β1β2γ3, α2β3γ2). Many drugs act at GABA_A receptors, including benzodiazepines, barbiturates, neurosteroids, local anesthetics, and anthelminitics. The type of subunits present determines if the drug acts as an agonist or an antagonist. The effect of barbiturates depends on the presence of GABA. In the absence of GABA, barbiturates act as activators of GABA_A receptors. Furosemide (3–300 μM) is a noncompetitive GABA_A antagonist. The anticonvulsant loreclazole potentiates GABA-induced currents. The structure and localization of many receptor subtypes have now been defined and some new members of the GABA_A gene family have been identified. Receptor subunits containing α5 were located in hippocampus, and drugs specific for these sites may
affect cognition. $\alpha_3$-Sites were found in limbic area and are targets for anxiolytic drugs, while $\alpha_1$ sites are widespread in the brain and are targets for sedative drugs.

The third plenary lecture, “Migraine, Ataxia and Epilepsy: A Challenging Spectrum of Genetically Determined Calcium Channelopathies,” was delivered by Michel D. Ferrari (Leiden University, Leiden, The Netherlands). Mutations of calcium channel genes were recently shown to be responsible for a number of genetic diseases involving the central nervous system. Mutations in the brain-specific, P/Q-type calcium channel $\alpha_1$-subunit gene on chromosome 19 are involved in familial hemiplegic migraine, episodic ataxia type 2, and chronic spinocerebellar ataxia type 6. This same gene may be responsible for the common form of migraine with or without aura. The tottering mouse syndrome, characterized by epilepsy and ataxia appears to be due to mutations in the mouse calcium channel in the $\alpha_1A$ subunit gene. Mutations in the $\gamma$-subunit gene may be involved in other genetic diseases of the central nervous system.

The last session of the symposium dealt with the clinical application of ion channel modulation. Andrew R. Blight (Univ. of North Carolina at Chapel Hill, NC) spoke about potassium-channel blockade in the treatment of spinal cord injury. According to Blight, the most widely studied potassium channel blocker is 4-aminopyridine (4-AP). 4-AP blocks primarily transient outward potassium current ($K_A$). Some functional benefits were obtained with 4-AP in patients with spinal cord injury and multiple sclerosis (MS). It is used in Europe as an analeptic. Animal studies indicate that 4-AP can improve synaptic transmission, restore conduction in damaged nerves, and increase acetylcholine release from the nerve endings. It was tried unsuccessfully in Alzheimer patients, but currently 4-AP is being developed for the treatment of MS by Elan Pharmaceuticals (holder of a patent for this indication) in collaboration with Accord Pharmaceuticals, NY. The major side effects include seizures and an increase in the excitability. C. P. Taylor (Parke-Davis Research, Ann Arbor, MI) discussed new indications for the anticonvulsant gabapentin. It is currently in Phase III clinical studies as an analgesic, and it was also active in animal models of anxiety. Gabapentin is effective in hyperalgesia models (e.g., Chung’s rat model) but not in the models of acute pain (e.g., hot plate). It is also effective in neurogenic inflammation and rat antigen arthritis. Gabapentin has at least two and possibly three important mechanisms of action. It was recently shown to bind with high affinity to the $\alpha_2\delta$ protein, an auxiliary subunit of voltage-gated calcium channels, to increase expression of the functional $\alpha_1$ subunit of L-type calcium channels and to reduce calcium currents in neurons. It reduced calcium entry in isolated pyramidal as well as sensory neurons, but had no effect on calcium currents in DRG or human dentate granule neurons. Gabapentin was also shown to inhibit dopamine- and K$^+$-induced norepinephrine release. Harald Sontheimer (Univ. of Alabama at Birmingham, AL) discussed glioma chloride channels as targets for the treatment of primary brain tumors. Sontheimer and his associates identified outwardly rectifying chloride currents in glioma cells from human tumors that were not present in cells from other tumors. These currents were antagonized by tetraethylammonium (TEA) and by chlorotoxin (Ctx), a 36 aminoacid peptide isolated from scorpion venom. In vitro Ctx slowed glioma cell migration and invasion. Fusion constructs of Ctx with saporin were lethal for glioma cells in vitro. Scott Bitner (Abbott Laboratories, Abbott Park, IL) reviewed the analgesic potential of cholinergic channel activation. As an analgesic epiba-
tidine is as effective as opioids. Abbott’s nicotinic agonist, ABT-594, is selective for nicotinic receptors containing the $\alpha_4\beta_2$ subunit and has reduced affinity for $\alpha_7$-containing subunits and, therefore, an improved safety profile in rodents. Drugs with high selectivity for $\alpha_4$ subunits are likely to be superior analgesics.

![ABT-594](image)

### POSTERS

The following posters were selected for a review in this report on the basis of their possible relevance to drug therapy. **G. Trube et al.** (F. Hoffmann La Roche, Basel, Switzerland) compared the effects of lubeluzole on cloned brain and cardiac sodium channels and erg-mediated potassium channels. The results indicate that lubeluzole is a more potent inhibitor of erg-potassium channels than sodium channels. It is, therefore, likely to prolong the Q interval in ECG, at doses below those required to inhibit sodium channels (an effect responsible for its neuroprotective activity). **Y. H. Chen et al.** (Glaxo Wellcome, Stevenage, Herts, U.K.) cloned and expressed four different voltage-gated sodium channel $\alpha$ subunits from human brain. Channels with either subunit conduct TTX-sensitive sodium currents. **F. Porreca et al.** (Univ. of Arizona, Tuscon, AZ) reported that the selective “knock-down” of PN3 protein in the dorsal root ganglion with antisense prevents and reverses thermal hyperalgesia and tactile allodynia caused by chronic nerve injury. S. Novakovic et al. (Roche Bioscience, Palo Alto, CA) reported that nerve demyelination leads to PN3 sodium channel accumulation and neuropathic pain syndrome. **F. C. Tortella et al.** (Walter Reed Army Research Inst., Washington, DC) described the expression pattern of TTX-sensitive and TTX-resistant sodium channels in rat brain after injury (MCAO). PN3 was not expressed in uninjured brain but was expressed in all injured areas at 24 h after injury. **E. Tzoumaka et al.** (Roche Bioscience, Palo Alto, CA) studied distribution of the sodium channel isoform PN5/NaN/SNS2 in the brain and spinal cord of normal rats and rats subjected to chronic constriction injury of the sciatic nerve. In normal animals this isoform was predominantly expressed in small DRG neurons, superficial laminae of the dorsal cord, as well as in the cerebellum and brainstem, but not in the sciatic nerve. After constriction injury of the sciatic nerve, PN5 protein was upregulated in the sciatic nerve of sham operated as well as of animals with the constricted sciatic nerve. The widespread distribution of this sodium channel isoform suggested its physiological importance. **Victor I. Ilyin et al.** (CoCensys, Inc., Irvine, CA) described the antiallodynic and antihyperalgesic effects of Co 102862 [4-(4-fluorophenoxy) benzaldehyde semicarbozone] in a rat model of peripheral neuropathy. Concentrations of Co 102862 greater than 3 $\mu$M inhibited sodium current in HEK cells transfected with the hSkM1
sodium channel α-subunit. In comparison with lamotrigine, phenytoin, and carbamazepine, Co 102862 has a higher affinity, faster association rate, and slower re-priming rate at the sodium channel. At doses of 1.25 to 10 mg/kg p.o., Co 102862 reversed tactile allodynia in the rat model of peripheral neuropathy. At doses of 2.5 to 5.0 mg/kg p.o., it reversed neuropathy-induced mechanical hyperalgesia. The drug was recommended for clinical use in the treatment of neuropathic pain.

K. R. Gogas et al. (Roche Bioscience, Palo Alto, CA) developed RS-132943 [(R,S)-3-(4-bromo-2,6-dimethylphenoxy)methyl]-1-methylpiperidine hydrochloride], a novel sodium channel blocker for the treatment of neuropathic pain. Most pharmacological studies were performed with the S-isomer of RS-132943. The isomer reversed tactile allodynia in the rat spinal nerve ligation model with an ED50 = 377 mg/kg p.o. It antagonized thermal hyperalgesia in the rat sciatic nerve constriction model with an ED50 = 16 mg/kg p.o. It was effective at lower doses when administered b.i.d. for 5 days. No overt behavioral effects were observed at doses up to 600 mg/kg p.o.

A. R. Cantrell et al. (Univ. of Washington, Seattle, WA) reported that activation of the D1-like dopamine receptor reduces sodium current in isolated hippocampal neurons. This effect involves phosphorylation of the α-subunit of the sodium channel by cAMP-dependent protein kinase (PKA). The extent of dopamine receptor modulation is affected by holding the potential and activation of protein kinase C (PKC). M. B. Hepworth and R. D. Pinnock (Parke-Davis Neuroscience Research Centre at Cambridge Univ., Cambridge, U.K.) reported that gabapentin inhibits the bradykinin-induced sensitization of heat-activated currents in primary DRG neurons. This effect may possibly account for the antihyperalgesic action of gabapentin. C. Kozlowski et al. (Glaxo Wellcome, Stevenage, Hertfordshire, U.K.) studied alterations in the expression of TTX-resistant sodium channels (SNS1 and SNS2) mRNA in sensory neurons following peripheral inflammation and concluded that inflammation modulates the expression of these channels.

M. A. Hurle et al. (Univ. of Cantabria, Santander, Spain) studied the mechanism of nimodipine-induced supersensitivity to opioids in rats. Rats developed tolerance to
sufentanil (tail-flick test). This effect was associated with the loss of the ability of forskolin to stimulate cAMP production in the brain tissue. Nimodipine restored the sensitivity to sufentanil and the effectiveness of forskolin. A. Kreimeyer et al. (CNRS-UMR, Illkirch, France, Max Planck Inst., Frankfurt, Germany and Bearsden-Bio Inc., Aston, PA) synthesized a series of ligands for the NMDA-receptor glycine site (derivatives of L-701,324). Their biological activity was demonstrated in oocytes transfected with NR1 and NR2 subunits of NMDA receptors.

K. Carpenter et al. (Univ. College, London, U.K.) studied neuropathic pain using rats with the ligation of L5/6 sciatic nerves. Ketamine and memantine antagonized responses of spinal neurons to thermal and mechanical stimuli. They were more effective in rats with L5/6 nerve ligation than in sham-operated animals. The N-type calcium channel antagonist conotoxin MVIIA reduced the responses of spinal neurons to noxious stimuli. C. Ikonomidou and J. W. Olney (Humboldt Univ., Berlin, Germany, and Washington Univ., St. Louis, MO) reported that neurons with NMDA receptors depend on glutamate for survival during synaptogenesis. NMDA-receptor antagonists (phencyclidine, ketamine, and ethanol) can trigger apoptotic neurodegeneration in immature rats. The investigators found that exposure of immature rats to ethanol (to maintain blood levels at 200 mg/dL for 4 to 6 h) will cause massive neurodegeneration in many regions of the developing CNS.